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The plastic cobras: omics investigation of venom evolveability and allopatric variation

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Snake venom is a complex polygenic trait driven by selection and is reflective of local adaptation of snakes to different ecological niches. The advent in –omics technologies has greatly improved the profiling of snake venom compositions, thereby providing a solid platform for investigating the mechanistic details underlying the evolutionary process of venom variability. Applying a venomic approach that integrates reversed-phase HPLC, gel-electrophoresis and nano-ESI-LCMS/MS, we profiled and compared the venoms of three Asiatic cobra species sourced from different geographical localities of their native distribution (*Naja naja*: Pakistan, India, Sri Lanka; *Naja kaouthia*: Thailand, Malaysia, Vietnam; *Naja sumatrana*: Thailand, northern and southern Malaysia, Sumatran Indonesia). Variations in venom composition are evident at intra-generic and intra-specific levels for all three cobra species, particularly on the expression of key toxins, i.e. alpha-neurotoxins and cytotoxins that are essential for predation and digestion. Of note, the Pakistani *N. naja*, Thai *N. kaouthia* and Indonesian *N. sumatrana* possess the highest content of neurotoxins (20-50% total venom proteins, in varied long-chain or short-chain isoforms), and these correlate with their potent lethal effects (LD_{50} = 0.2-0.4 µg/g in mice) and the syndromic evolution, as well as antigenic responses to region-specific antivenom. Furthermore, Illumina HiSeq technology applied for comparative venom-gland transcriptomics of *N. kaouthia* has allowed the mapping of specific transcripts to toxins of significance, thereby correlating the toxin genotype to phenotypic venom variation. Our findings revealed that although most toxin genes are highly conserved, distinct differential gene expression was remarkable, indicating up-regulation of gene transcription for selected toxins, or enhanced mRNA degradation or lack of transcription due to pseudogenization of certain traits. The findings highlight the significance of the plasticity of key-toxin compositions in cobras as unique venom phenotypes, and provide critical insights intoenvenomation pathophysiology as well as the formulation of an effective pan-regional antivenom.

Keywords: snake venom, venomics, transcriptomics, venom variation, cobra, *Naja*